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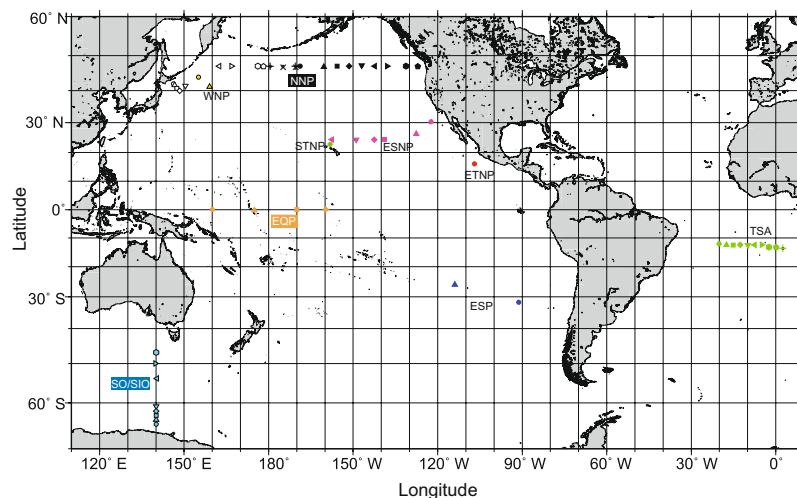
## Identifying the origin of nitrous oxide dissolved in deep ocean by concentration and isotopocule analyses

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Nitrous oxide ( $N_2O$ ) contributes to global warming and stratospheric ozone depletion. Although its major sources are regarded as bacterial or archaeal nitrification and denitrification in soil and water, the origins of ubiquitous marine  $N_2O$  maximum at depths of 100–800 m and  $N_2O$  dissolved in deeper seawater have not been identified. We examined  $N_2O$  production processes in the middle and deep sea by analyzing vertical profiles of  $N_2O$  concentration and isotopocule ratios, abundance ratios of molecules substituted with rare stable isotopes  $^{15}N$  or  $^{18}O$  to common molecules  $^{14}N^{14}N^{16}O$ , in the Atlantic, Pacific, Indian, and Southern oceans. Isotopocule ratios suggest that the  $N_2O$  concentration maxima is generated by *in situ* microbial processes rather than lateral advection or diffusion from biologically active sea areas such as the eastern tropical North Pacific. Major production process is nitrification by ammonia-oxidizing archaea (AOA) in the North Pacific although other processes such as bacterial nitrification/denitrification and nitrifier-denitrification also significantly contribute in the equatorial Pacific, eastern South Pacific, Southern Ocean/southeastern Indian Ocean, and tropical South Atlantic. Concentrations of  $N_2O$  below 2000 m show significant correlation with the water mass age, which supports an earlier report suggesting production of  $N_2O$  during deep water circulation. Furthermore, the isotopocule ratios suggest that AOA produce  $N_2O$  in deep waters. These facts indicate that AOA have a more important role in marine  $N_2O$  production than bacteria and that change in global deep water circulation could affect concentration and isotopocule ratios of atmospheric  $N_2O$  in a millennium time scale.

The oceans are estimated as the third largest source of atmospheric  $N_2O$  after natural soil and agriculture<sup>1</sup>. Oceans are also a large reservoir of this greenhouse and ozone-depleting gas because  $N_2O$  has seawater solubility as high as  $CO_2$ <sup>2</sup>. Moreover, oversaturation of  $N_2O$  is often found at depths below the surface layer (<ca. 100 m depth)<sup>3</sup>. In many ocean areas, the  $N_2O$  concentration increases with depth and shows a maximum at 100–800 m depth. The highest concentration is found in the eastern tropical North/South Pacific (ETNP/ETSP)<sup>4</sup>. Based on these facts, it has been proposed that  $N_2O$  produced in the ETNP/ETSP, where biological activities are very high because of upwelling of nutrient rich water, is globally transported by isopycnal movement of seawater<sup>4</sup>. However, one earlier report describes that  $N_2O$  concentrations in the water deeper than 2000 m show positive correlation with the age of the water with respect to global circulation<sup>5</sup>, suggesting that  $N_2O$  production might also occur in the deep water. Although deep waters do not contact with the atmosphere, such  $N_2O$  oversaturation is expected to affect ocean-to-atmosphere flux of  $N_2O$  on a longer time scale by diffusive transport or by change in global ocean

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**Figure 1.** Map showing seawater sampling stations. In this work, observations in the northern North Pacific (NNP), equatorial Pacific (EQP), and Southern Ocean/southeastern Indian Ocean (SO/SIO) were conducted. Data in other regions were compiled from previous studies. See also Table S1. Each symbol corresponds to the symbol presented in Figs 2 and 3.

circulation. Nevertheless, the origin and production mechanisms of the  $N_2O$  maximum and the  $N_2O$  in deep water remain uncertain.

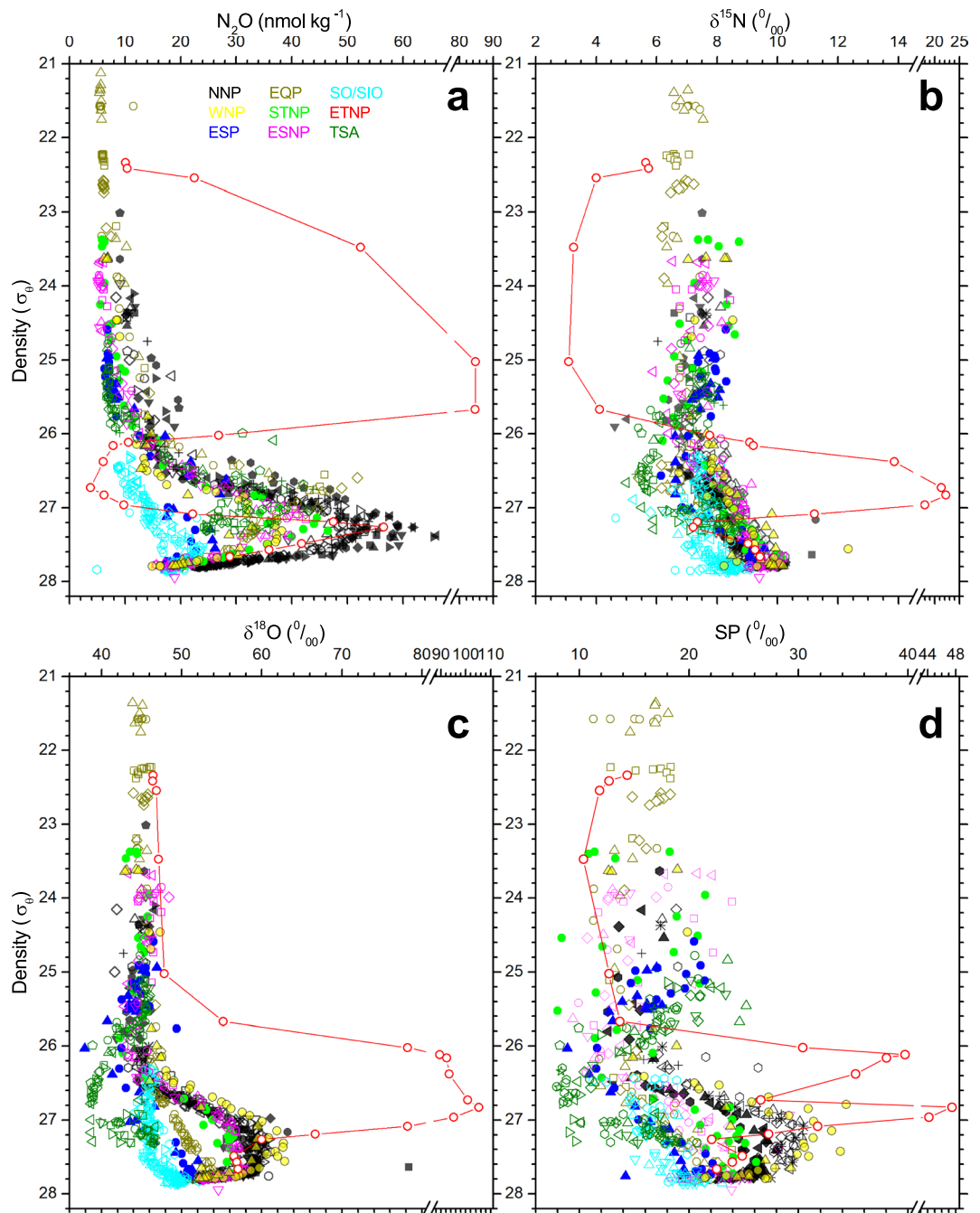
Isotopocule ratios ( $\delta^{15}N$ ,  $\delta^{18}O$ , and SP,  $^{15}N$ -site preference in NNO molecule) of  $N_2O$  are useful parameters to identify the origin and production–consumption processes of  $N_2O$  because they depend on the isotopic ratios in precursors and isotope effects of chemical or biochemical reactions<sup>6</sup>. Reportedly, the magnitudes of isotope effects on nitrogen during  $N_2O$  production as byproduct of nitrification by ammonia oxidizing bacteria (AOB)<sup>7,8</sup> and ammonia oxidizing archaea (AOA)<sup>9,10</sup> differ. Moreover, SP values of  $N_2O$  produced by AOB via hydroxylamine ( $NH_2OH$ )<sup>9,11</sup> and by AOA<sup>9,10,12</sup> are distinct from the values of  $N_2O$  produced by AOB via nitrite ( $NO_2^-$ )<sup>8,11,13,14</sup> or by denitrification<sup>11,15</sup> (Supplementary Fig. S1, Table S2). When  $N_2O$  is partially reduced to  $N_2$  in denitrification, all isotopocule ratios increase<sup>16–20</sup>. Although the isotope effect during  $N_2O$  reduction varies among bacterial species or pure culture and community incubations, consistent relations have been found between isotope effects on N, O, and SP<sup>16</sup>.

Several earlier studies have analyzed vertical profiles of concentration and isotopocule ratios of  $N_2O$  in the ocean and have investigated production–consumption processes specific to the depths or sites<sup>17,21–25</sup>. Nevertheless, it remains unclear whether  $N_2O$  is produced *in situ* or transported from other regions because existing data do not cover the wide range of oceanic setting such as circulation age of deep seawater. This report describes new isotopocule analyses of  $N_2O$  in the northern North Pacific (NNP), equatorial Pacific (EQP), and Southern Ocean and southeastern Indian Ocean (SO/SIO) where younger, medium, and older water respectively exist in deep layers (Fig. 1). The respective results are compared with existing observations to ascertain the origin of  $N_2O$  from the perspective of global deep water circulation.

### Vertical Profiles of Dissolved $N_2O$

In the surface layer, concentrations of  $N_2O$  measured in the eight regions except the eastern tropical North Pacific (ETNP) are as low as those expected under the dissolution equilibrium between atmosphere and seawater ( $6\text{--}8\text{ nmol kg}^{-1}$ ). However, they increase with depth and reach a maximum ( $25\text{--}65\text{ nmol kg}^{-1}$ ) (Fig. 2a). The depth of the maximum is 100–400 m in the tropical South Atlantic (TSA), SO, and EQP, and 400–1500 m in other regions. Those values correspond to almost identical seawater density (potential density anomaly,  $\sigma_\theta$ , of about 27) (Supplementary Table S1). Below the maximum, the  $N_2O$  concentration decreases with depth. It reaches  $15\text{--}40\text{ nmol kg}^{-1}$  in the deep layer ( $>2000\text{ m}$  depth or  $\sigma_\theta = 27.7\text{--}27.8$ ). In the ETNP, two concentration maxima exist at 60 m ( $\sigma_\theta = 25.0$ ) and 800 m ( $\sigma_\theta = 27.3$ )<sup>17</sup>.

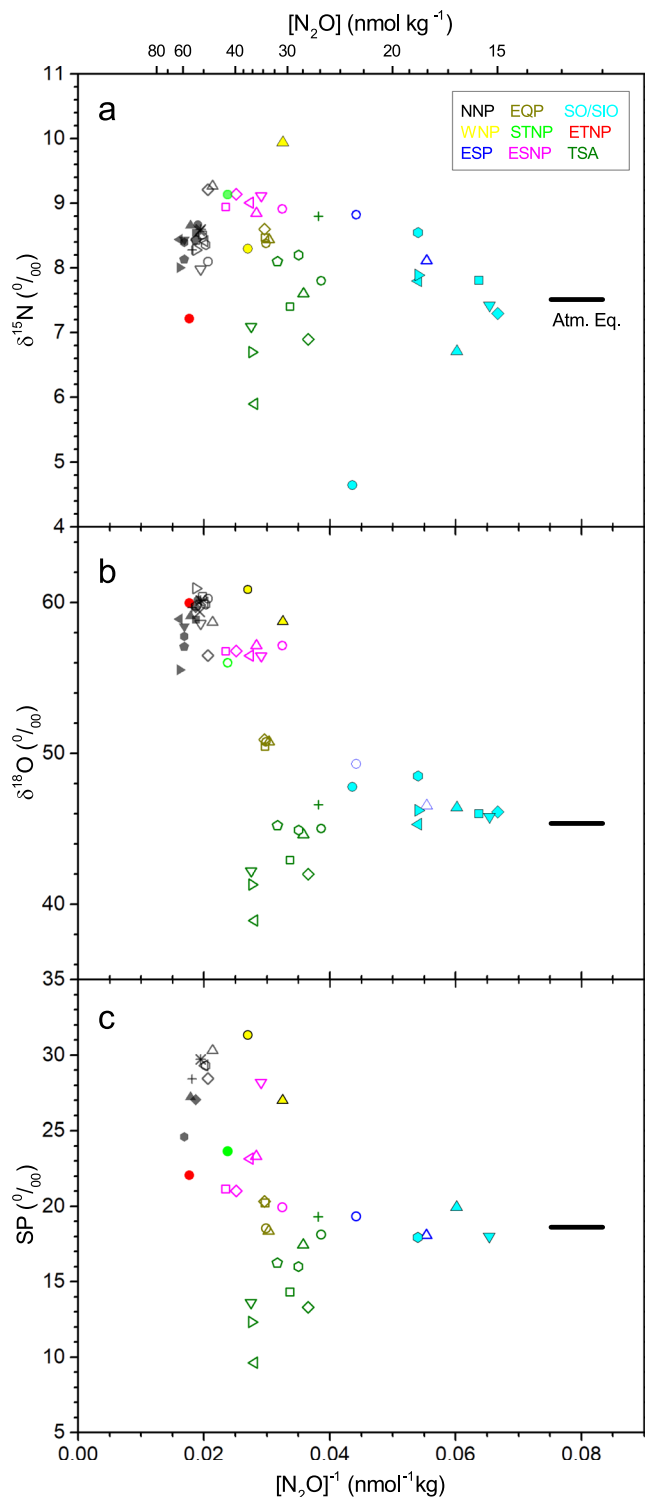
Each of the three independent isotopocule ratios shows a unique profile that is different from that of concentration (Fig. 2b–d). The bulk (or average) nitrogen isotope ratio ( $\delta^{15}N^{\text{bulk}}$ ) in the eight regions except ETNP shows no vertical gradient from the surface to the depth corresponding to  $\sigma_\theta \cong 25$ . Then it decreases slightly with depth, showing a minimum value at  $\sigma_\theta \cong 26$ . Below the small minimum, it increases monotonically with depth and reaches its maximum (9–10‰) at the bottom layer. In the ETNP, it shows a minimum and a maximum respectively corresponding to the shallow and deep concentration maxima. The oxygen isotope ratio ( $\delta^{18}O$ ) and  $^{15}N$ -site preference (SP) also show almost constant values from the surface to the depth above the concentration maximum ( $\sigma_\theta \cong 26$ ) in the eight regions. In deeper water, however, their vertical profiles are markedly different among oceanic regions. Although  $\delta^{18}O$  and SP respectively show a parallel increase and decrease with concentration in the North Pacific stations, they exhibit a monotonic increase in EQP, SO/SIO, and ESP. In TSA, they show minima at the concentration maximum.



**Figure 2.** Vertical distribution of concentrations and isotopic ratios of  $N_2O$  in nine oceanic regions. (a–d) Concentration (a),  $\delta^{15}N^{bulk}$  (b),  $\delta^{18}O$  (c), and SP (d). Symbol colors represent regions. Vertical axis shows potential density anomaly instead of depth, in order to compare the  $N_2O$  profiles obtained from regions with different physical structures. When samples were collected at several stations in a region, the symbol type was changed accordingly: NNP, northern North Pacific; EQP, equatorial Pacific; SO/SIO, Southern Ocean/southeastern Indian Ocean; WNP, western North Pacific; STNP, subtropical North Pacific; ETNP, eastern tropical North Pacific; ESP, eastern South Pacific; ESNP, eastern subtropical North Pacific; TSA, tropical South Atlantic. See Table S1 for data sources.

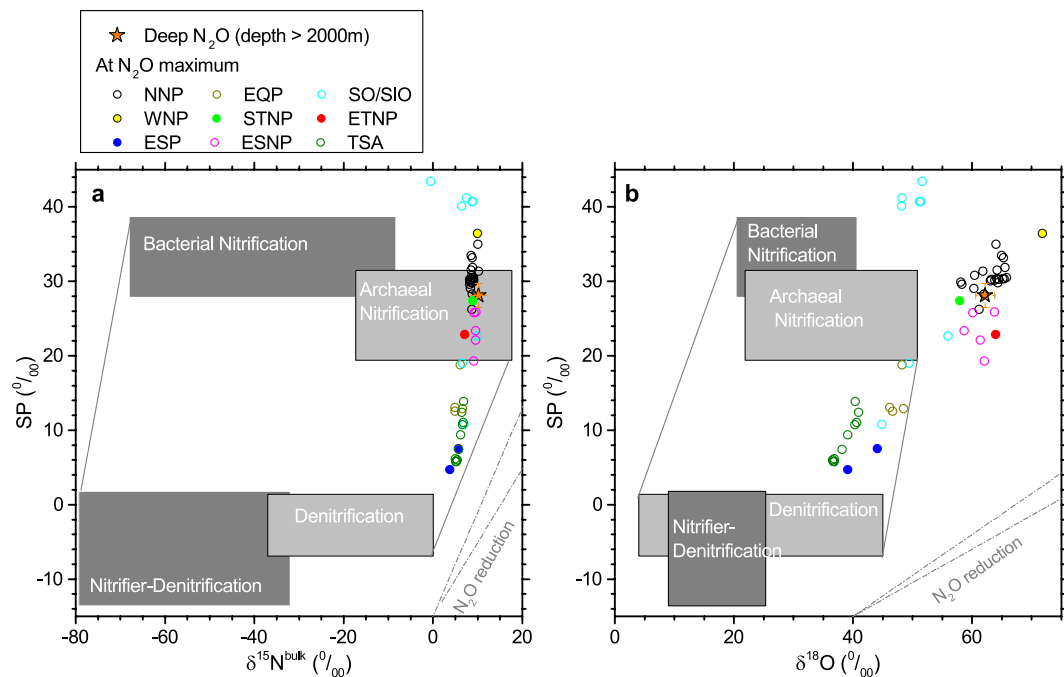
### Origin of $N_2O$ at Concentration Maximum

Isotopic signatures of  $N_2O$  at density level of  $\sigma_\theta = 27.3$  which corresponds to concentration maximum in ETNP show systematic difference between oceanic regions (Fig. 3). For example, in spite of similarity in water mass property (temperature and salinity, data not shown) between NNP and WNP and between EQP and ETNP, they are distinguished each other. Variations of isotopic ratios are caused by (1) mixing of  $N_2O$  with different isotopic signatures such as  $N_2O$  produced *in situ*, advected from other regions, and dissolved atmospheric  $N_2O$  or (2) decomposition of  $N_2O$  during which the remaining  $N_2O$  is isotopically fractionated<sup>6</sup>. Based on a global



**Figure 3.** Comparison of isotopocule ratios of N<sub>2</sub>O at concentration maximum in nine oceanic regions. (a–c) Relations between inverse concentration and δ<sup>15</sup>N<sup>bulk</sup> (a), δ<sup>18</sup>O (b), and SP (c). Colors and types of symbols are as shown in Fig. 2. Horizontal solid lines show values of dissolved N<sub>2</sub>O when the seawater is equilibrated with the atmosphere.

distribution of N<sub>2</sub>O concentration, it has been proposed that N<sub>2</sub>O produced in the eastern tropical Pacific is exported to other regions in the Pacific by lateral or isopycnal advection<sup>4</sup>. If this is the case, and if we consider mixing of two endmembers, namely, N<sub>2</sub>O produced in the ETNP and background N<sub>2</sub>O from the atmosphere, the isotopic data points for the regions outside the ETNP is expected to fall on the mixing line in isotope–reciprocal concentration plot. However, the results show that mixing between N<sub>2</sub>O in the ETNP and N<sub>2</sub>O in the water equilibrated with the atmosphere cannot explain the observed isotopocule ratios in the eight regions (Fig. 3). In



**Figure 4.** Comparison of isotopocule ratio of the excess N<sub>2</sub>O produced at concentration maximum (circles) and deeper seawater (orange star). (a,b) Relation between  $\delta^{15}\text{N}^{\text{bulk}}$  and SP (a) and the relation between  $\delta^{18}\text{O}$  and SP (b). Rectangles respectively show ranges expected when N<sub>2</sub>O is produced by the indicated microbial processes (See Supplementary Information). Broken lines show range of the slope expected for increase in isotopocule ratios during partial reduction by denitrification.

other words, the isotopocule ratios for excess N<sub>2</sub>O at N<sub>2</sub>O maxima in the eight regions, as calculated assuming isotopic mass balance between observed and background N<sub>2</sub>O, are distinct from those obtained at ETNP (Fig. 4 and Table S1).

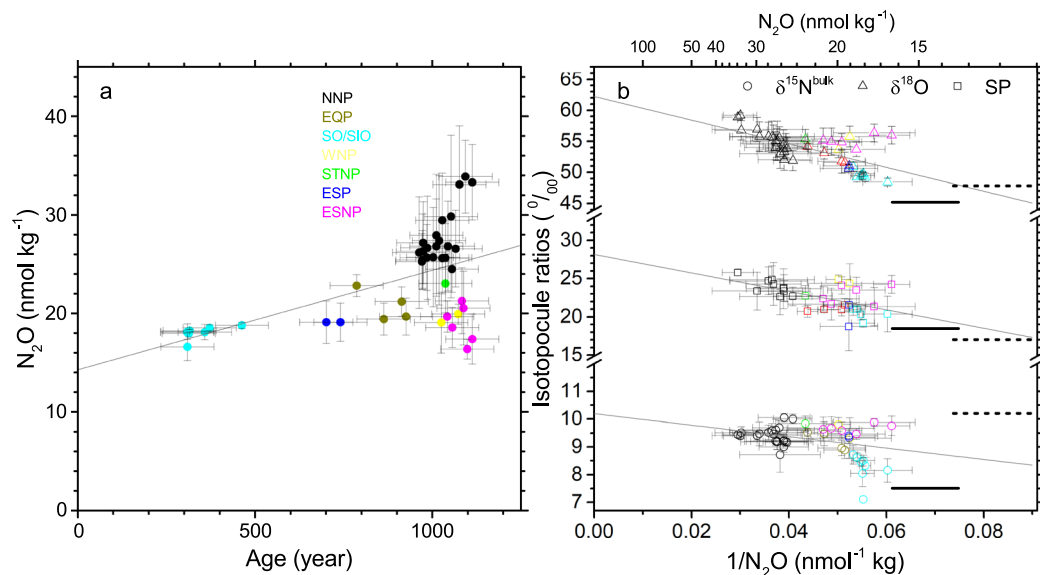
In Fig. 3, N<sub>2</sub>O maxima in NNP could be regarded as an alternative endmember because of its high concentration. However, lateral advection of N<sub>2</sub>O from NNP is also improbable for the following reasons. First, the distribution of data obtained in other regions still cannot be explained consistently by the isopycnal mixing in each panel of Fig. 3. Second, vertical profiles of temperature and salinity at the stations in this work do not show discontinuous feature which is expected if different water mass is advected laterally.

Contribution from partial reduction of N<sub>2</sub>O (the second factor noted above) is also unlikely because all the isotopic signatures should be increased with decreasing concentration and because nitrate reduction, which is the first step of denitrification and prerequisite for N<sub>2</sub>O reduction, is not prominent in the observed oxic water columns. Therefore, we infer that the N<sub>2</sub>O maximum is a result of *in situ* production rather than advection from other regions. Possible production mechanisms are discussed below together with those for deep water N<sub>2</sub>O.

### Origin of N<sub>2</sub>O in Deep Layer

The concentration of N<sub>2</sub>O averaged for water below 2000 m depth shows a positive correlation with the circulation age of seawater estimated from the <sup>14</sup>C content<sup>26</sup> (Fig. 5a). Bange and Andreae found a similar relation by compiling 56 observations from the North/South Atlantic, North/South Indian, and North Pacific oceans<sup>5</sup>. Based on insignificant fluxes estimated for hydrothermal or sediment N<sub>2</sub>O, they concluded that N<sub>2</sub>O is produced in the deep ocean mainly by nitrification. The slope of the regression line in Fig. 5a ( $(1.0 \pm 0.2) \times 10^{-2} \text{ nmol kg}^{-1} \text{ yr}^{-1}$ ) is comparable to their reported value of  $(5.7 \pm 1.0) \times 10^{-3} \text{ nmol L}^{-1} \text{ yr}^{-1}$  and the y-intercept ( $14.3 \pm 2.2 \text{ nmol kg}^{-1}$ ) agrees with the earlier report ( $13.1 \text{ nmol kg}^{-1}$ )<sup>5</sup>. Here we show that isotopocule ratios also increase with the circulation age. When plotted against inverse N<sub>2</sub>O concentration, they are distributed on a line that passes ranges of surface water that is saturated with the atmosphere (Fig. 5b). This confirms that N<sub>2</sub>O is added during the circulation of deep seawater after losing contact with the atmosphere, and the following isotopocule ratios of the produced N<sub>2</sub>O are obtained as y-intercepts of the regression lines:  $10.2 \pm 0.4\%$ ,  $62.2 \pm 1.6\%$ , and  $28.1 \pm 1.6\%$ , respectively, for  $\delta^{15}\text{N}^{\text{bulk}}$ ,  $\delta^{18}\text{O}$ , and SP.

A closer look at Fig. 5a and Supplementary Table S1 reveals systematic difference in N<sub>2</sub>O concentration between NNP, STNP, WNP, and ESNP. Since it has been suggested that deep water circulates from the South Pacific to these north Pacific regions via different routes<sup>27,28</sup>, N<sub>2</sub>O production rate during the circulation might vary with the pathways. For instance, dissolved oxygen concentration in the deep layer is lowest in NNP<sup>29</sup>, which could explain the higher N<sub>2</sub>O concentration in NNP than other North Pacific regions because N<sub>2</sub>O production is enhanced under low oxygen condition<sup>12,30</sup>. Additional factor that could partly explain the positive deviations of NNP data from the fitted line is underestimation of age for the NNP waters (Matsumoto, personal communication).



**Figure 5.** Concentration and isotopocule ratios of  $N_2O$  for deep water (depth >2000 m) in nine oceanic regions and their relation with circulation age of seawater. **(a,b)** Relation between circulation age and  $N_2O$  concentration **(a)** and relation between inverse concentration and isotopocule ratios of  $N_2O$  **(b)**. Each symbol denotes the average for the deep water at each station. Error bars for concentration and isotopocule ratios show one standard deviation while those for age are reported estimated error<sup>26</sup>. Solid and broken horizontal lines in panel b respectively show the values expected when the surface water in the subduction region is equilibrated with modern and preindustrial atmosphere<sup>31</sup>. Correlation coefficients of regression lines and probabilities of null hypothesis in ANOVA are as follows. In panel a,  $r = 0.55$ ,  $p = 1.3 \times 10^{-4}$ ; in panel b,  $\delta^{15}N^{bulk}$ ,  $r = -0.38$ ,  $p = 1.3 \times 10^{-2}$ ;  $\delta^{18}O$ ,  $r = -0.65$ ,  $p = 2.3 \times 10^{-6}$ ; SP,  $r = -0.56$ ,  $p = 1.4 \times 10^{-3}$ .

In consideration of millennium scale of deep water circulation, concentration and isotopic signature of  $N_2O$  initially existed in the water mass before subduction do not equal to the present values. Solid and broken horizontal lines in Fig. 5b respectively show the values for the surface water in the subduction region (off Greenland and off Antarctica) which is equilibrated with modern and preindustrial atmosphere<sup>31</sup>. The negative deviation of the SO/SIO data might indicate that relatively younger water mass in this region is affected by the mixing of modern surface water during its advection.

### Production Mechanisms of $N_2O$ in Concentration Maximum and Deep Layer

We compare the isotopocule ratio values of excess  $N_2O$  at concentration maximum and accumulated  $N_2O$  during deep water circulation with those values reported or estimated for currently known biological processes of oceanic  $N_2O$  (Fig. 4, Supplementary Table S4). The ranges of  $\delta^{15}N^{bulk}$  and SP values for the  $N_2O$  maximum at most of the stations in the North Pacific and the values for deep  $N_2O$  overlap with that of  $N_2O$  produced by archaeal nitrification conducted by ammonia-oxidizing archaea (AOA)<sup>9,10,12</sup> (Fig. 4a). At several stations in the North Pacific and SO/SIO, SP values of  $N_2O$  at maximum concentration are larger than the range of AOA-derived  $N_2O$ , suggesting additional contribution from bacterial nitrification. In the Atlantic and at several stations in the equatorial/South Pacific and SO/SIO,  $N_2O$  maxima are accompanied by lower SP values, which indicates significant role of nitrifier-denitrification/denitrification. In contrast,  $\delta^{18}O$  values obtained for both  $N_2O$  maximum and deep water  $N_2O$  are higher than that of AOA by 5–20‰ (Fig. 4b). If  $N_2O$  produced by AOA or bacterial processes is further reduced by denitrification,  $\delta^{18}O$  of residual  $N_2O$  increases due to isotope fractionation<sup>16–20</sup>. However, the parallel increases in  $\delta^{15}N^{bulk}$  and SP expected for  $N_2O$  reduction (slope of broken lines in Fig. 4) are not prominent especially for  $\delta^{15}N^{bulk}$  and it is unlikely that  $N_2O$  reduction occurs in oxic seawater because  $N_2O$  reduction has been found only in anoxic environment such as ETNP or Arabian Sea<sup>17,32</sup>. Therefore, the discrepancy of  $\delta^{18}O$  might be explained by variation of oxygen isotope effects during  $N_2O$  production by AOA. This should be tested using further laboratory studies with several species of AOA. Reported oxygen isotope fractionation during the incorporation of  $O_2$  into  $N_2O$  is  $-2.2$  to  $13.2\%$  in two marine and five soil archaeal species/isolates<sup>9,10</sup>. Those figures might become larger if the reaction proceeds with the equilibrium step because O-isotope exchange equilibrium fractionation between  $N_2O$  and  $O_2$  is calculated theoretically as about  $20\%$  at  $0$ – $10^\circ C$ <sup>33</sup>.

Several reports have described the dominance of AOA in nitrification in the ocean. Archaeal *amoA* gene abundance was positively correlated with potential  $NH_3$  oxidation rate and  $N_2O$  concentration in the upper oxycline of the eastern tropical South Pacific<sup>34</sup>. In the oxygen minimum zone (OMZ) of the eastern tropical North Atlantic, comparable patterns of abundance and expression of archaeal *amoA* genes and  $N_2O$  co-occurred<sup>12</sup>. Our results suggest that AOA play a major role not only in the OMZ of tropical ocean but also in the OMZ in temperate and subarctic ocean areas and deeper waters. The average rate of  $N_2O$  production during deep water circulation estimated from the relation between  $N_2O$  concentration and circulation age of seawater is  $28 \text{ fmol L}^{-1} \text{ d}^{-1}$ ,

which is comparable to the rate determined experimentally with pure culture of marine AOA *Nitrosopumilus maritimus* SCM1 ( $4.4 \times 10^{-4}$ – $2.4 \times 10^{-2}$  f mol d<sup>-1</sup> cell<sup>-1</sup> under oxygen concentrations of 203–34 μmol L<sup>-1</sup>)<sup>12</sup> if one assumes cell density of AOA in deep waters as 10<sup>4</sup> cells L<sup>-1</sup>.

Our results suggest that ubiquitous N<sub>2</sub>O maximum in the middle layer in the North Pacific is formed by *in situ* production (mainly by AOA) rather than advection from the eastern tropical Pacific and that N<sub>2</sub>O is also produced by AOA and accumulated during the global deep water circulation. If one simply assumes that the N<sub>2</sub>O dissolved in the oldest deep water in NNP (34 nmol kg<sup>-1</sup>) is ultimately released to the atmosphere by the circulation driven by seawater subduction in the polar regions ( $3 \times 10^7$  m<sup>3</sup> s<sup>-1</sup>)<sup>35</sup>, magnitude of this deep N<sub>2</sub>O source is estimated at up to 1 Tg N yr<sup>-1</sup> compared to the estimated surface oceanic source of 3.8 Tg N yr<sup>-1</sup> which seems to be based on limited surface observations. This implies that change in global deep water circulation could affect concentration and isotopocule ratios of atmospheric N<sub>2</sub>O in a millennium time scale.

## Methods

Seawater samples were collected at 21 stations in the North Pacific (40–47°N, 146°E–127°W) during July–August 2007 and 4 stations in the equatorial Pacific (0°N, 160°E–158°W) in January 2003 during the MR07-04 and MR02-K06 cruises, respectively, of R/V *Mirai* (JAMSTEC, Japan) and at 8 stations in the Southern Ocean/southeastern Indian Ocean (47–65°S, 140°E) in Jan 2002 during the KH01-3 cruise of R/V *Hakuho-maru* (JAMSTEC, Japan). At each station, samples were taken at 15–28 depths in the range of 0–6000 m using 12 L Niskin bottles mounted on a conductivity-temperature-depth Rosette sampler. Each sample was collected in a 230 mL glass vial followed by addition by 1 mL saturated HgCl<sub>2</sub> solution for sterilization and by sealing with a butyl rubber stopper. Each was then preserved at 4 °C in the dark.

Concentration and isotopocule ratios of N<sub>2</sub>O were measured using a gas chromatograph – isotope ratio mass spectrometer (GC-IRMS) equipped with a gas extraction and cryogenic concentration unit as described elsewhere<sup>17,36</sup>. Isotopocule ratios  $^{15}R^{\alpha}$  ( $= [^{14}\text{N}^{15}\text{N}^{16}\text{O}]/[^{14}\text{N}^{14}\text{N}^{16}\text{O}]$ ),  $^{15}R^{\beta}$  ( $= [^{15}\text{N}^{14}\text{N}^{16}\text{O}]/[^{14}\text{N}^{14}\text{N}^{16}\text{O}]$ ), and  $^{18}R$  ( $= [^{14}\text{N}^{14}\text{N}^{18}\text{O}]/[^{14}\text{N}^{14}\text{N}^{16}\text{O}]$ ) are expressed as delta values as defined below<sup>37</sup>.

$$\delta X = (R - R_{\text{standard}})/R_{\text{standard}} \quad (1)$$

Therein, X denotes  $^{15}\text{N}^{\alpha}$ ,  $^{15}\text{N}^{\beta}$ , or  $^{18}\text{O}$ ; R represents  $^{15}R^{\alpha}$ ,  $^{15}R^{\beta}$ , or  $^{18}R$ ;  $R_{\text{standard}}$  means  $[^{15}\text{N}^{14}\text{N}]/[^{14}\text{N}^{14}\text{N}]$  of atmospheric N<sub>2</sub> or  $[\text{H}_2^{18}\text{O}]/[\text{H}_2^{16}\text{O}]$  of Vienna Standard Mean Ocean Water. In this report, we use δ value for bulk N and  $^{15}\text{N}$ -site preference instead of  $\delta^{15}\text{N}^{\alpha}$  and  $\delta^{15}\text{N}^{\beta}$ .

$$\delta^{15}\text{N}^{\text{bulk}} = (\delta^{15}\text{N}^{\alpha} + \delta^{15}\text{N}^{\beta})/2, \quad (2)$$

$$\text{SP} = \delta^{15}\text{N}^{\alpha} - \delta^{15}\text{N}^{\beta}. \quad (3)$$

The typical analytical precision (1σ) is 1% for concentration, and 0.2‰, 0.4‰, and 0.9‰, respectively, for  $\delta^{15}\text{N}^{\text{bulk}}$ ,  $\delta^{18}\text{O}$ , and SP.

Isotopocule ratios of excess N<sub>2</sub>O at its maximum and those of N<sub>2</sub>O produced *in situ* during deep water circulation were estimated by assuming the mixing of two end members.

$$C_{\text{obs}} = C_{\text{pro}} + C_{\text{atm}} \quad (4)$$

$$\delta_{\text{obs}}C_{\text{obs}} = \delta_{\text{pro}}C_{\text{pro}} + \delta_{\text{atm}}C_{\text{atm}} \quad (5)$$

Equations (4) and (5) respectively describe the mass balance of light and heavy N<sub>2</sub>O molecules;  $C_{\text{obs}}$ ,  $C_{\text{pro}}$ ,  $C_{\text{atm}}$  respectively denote the observed, produced, and atmospheric equilibrium concentrations;  $\delta_{\text{obs}}$ ,  $\delta_{\text{pro}}$ , and  $\delta_{\text{atm}}$  are the respective isotopocule ratios. By eliminating  $C_{\text{pro}}$  from Eqs (4) and (5), we obtain the following:

$$\delta_{\text{pro}} = (\delta_{\text{obs}}C_{\text{obs}} - \delta_{\text{atm}}C_{\text{atm}})/(C_{\text{obs}} - C_{\text{atm}}) \quad (6)$$

The circulation age of deep seawater at each observational station was calculated from the objectively mapped circulation <sup>14</sup>C age below 1500 m in the literature<sup>26</sup> using “2D estimation” tool of Ocean Data View software<sup>38</sup>.

## Data Availability

Data presented in Fig. 2 are available upon request, and will be archived at <http://www.godac.jamstec.go.jp/darwin/e> or <https://www.jodc.go.jp/jodcweb/index.html>.

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## Author Contributions

O.Y. and S.W. arranged and conducted seawater sampling. A.F. and H.Y. collected seawater samples and conducted N<sub>2</sub>O analyses. S.T. and N.Y. made data analyses and wrote the manuscript.

## Additional Information

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